

## **A. FRONT COVER/TITLE PAGE**

TITLE OF RESEARCH PROJECT:

EFFECTS OF QUALITY COMPOSTS AND OTHER ORGANIC AMENDMENTS  
AND THEIR HUMIC AND FULVIC ACID FRACTIONS ON THE  
GERMINATION AND EARLY GROWTH OF  
SLICKSPOT PEPPERGRASS (*LEPIDIUM PAPILLIFERUM*) AND SWITCHGRASS  
IN VARIOUS EXPERIMENTAL CONDITIONS

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NAME OF CONTRACTOR: UNIVERSITA' DI BARI

AWARD/CONTRACT NO: W911NF-08-1-0076-P00001

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### **3<sup>rd</sup> and 4<sup>th</sup> INTERIM REPORTs**

REPORT PERIOD: 1 April 2009-31 March 2010

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## B. BODY OF THE REPORT

### (1) SCIENTIFIC WORK DONE DURING THE REPORTING PERIOD

#### 1. Germination and early-growth experiments of slickspot peppergrass (*Lepidium papilliferum*) seeds

##### *1.1. Experimental*

##### *1.1.1. Germination*

During the 3<sup>rd</sup> and 4<sup>th</sup> semester (2<sup>nd</sup> year) of this research, a series of experiments was performed in order to evaluate the effects of different dissolved organ matter (DOM), or fulvic acid, samples obtained from composts under study on the germination of the native American species slickspot peppergrass (*Lepidium papilliferum*).

Briefly, the three DOM fractions were previously isolated from three selected composts, a green compost (GC-DOM), a mixed compost (MC-DOM) and a green coffee compost (GCC-DOM). The main physical and chemical properties of the three compost samples and their DOM fractions were shown and described in detail in the first report of this Project. These are summarized in Table 1.

Since the germination trials carried out during the 2<sup>nd</sup> semester of this Project, which results were shown and described in the 2<sup>nd</sup> report, produced an appreciable germination performance, in these new experiments the same procedure was applied using DOM samples. Sixty (60) seeds of slickspot peppergrass for each treatment were previously scraped dry with fine sand paper, soaked in distilled water for 12 h, and pierced with steel needle before starting germination experiments.

The treatments were prepared by adding 3 g of agar in 200 ml of Nitch nutrient solution alone (control) or in the presence of each DOM sample at dilutions of 1:10, 1:5 and 1:2 (DOM:nutrient solution). All the treatment solutions were sterilized by autoclaving at 120 °C for 15 minutes. After cooling until about 55 °C, the substrates were poured in Petri dishes where they were let to completely cool to room temperature, and solidify. Twenty (20) seeds of slickspot peppergrass pretreated as previously described were placed in Petri dishes containing the various substrates and kept in a thermostated chamber in the following conditions: (a) photoperiod of 8-h; (b) temperature of 18 °C during the illumination period and 10 °C during the dark period; and (c) illumination of about 2000 lux. After 7 days, germinated seeds were counted and primary root and

shoot lengths were measured. Since seed germination continued in the days after transplanting, germinated seeds were counted also after 15 days.

All experiments were triplicated and data obtained were statistically analyzed by one-way analysis of variance (ANOVA) at 95% and 99% confidence levels, and the mean values obtained for the different treatments were separated by using the least significant difference test (LSD).

#### *1.1.2. Early-growth*

The early-growth experiments were started on germinated seeds (seedlings) of slickspot peppergrass collected from the Petri dishes immediately after the end of the germination trials described in section *1.1.1*. The seedlings were transplanted into new Petri dishes containing the same treatments described for germination experiments in section *1.1.1*. i.e., Nitch nutrient solution in agar in the absence (control) or presence of each DOM samples at dilutions of 1:10, 1:5, and 1:2.

The Petri dishes were then placed in a thermostated chamber where seedlings were allowed to grow for a period of 30 days in the following conditions: (a) photoperiod of 12-h; (b) temperature of 18 °C during the illumination period and 10 °C during the dark period; and (c) constant humidity of 65% and (d) illumination of about 5000 lux. After 15 days growth, and at the end of the experiments (30 days), seedling root and shoot lengths were measured. Because of the very little amount of fresh plant material, the fresh and dry weights of shoots and roots could not be measured. All experiments were conducted in three replicates and data obtained were statistically analyzed by ANOVA at 95% and 99% confidence levels, and the mean values obtained for the different treatments were separated by using the least significant difference test (LSD). At the end of early-growth experiments (30 days), the most vigorous seedlings of slickspot peppergrass for each treatment were transplanted into plastic pots containing a commercial growing substrate in order to continue the growth up to 60 days and 6 months. The substrate is a mixture of an extensively decomposed peat (frozen black peat), a slightly to medium decomposed peat (white peat), sand and NPK-fertilizer. In particular, the characteristics of the substrate (German origin) are the following: (a) pH (CaCl<sub>2</sub>) 5.2-6.0; (b) salt content (H<sub>2</sub>O) < 1.5 g/l; (c) 120-210 mg/l N; (d) 120-210 mg/l P<sub>2</sub>O<sub>5</sub>; and (e) 170-300 mg/l K<sub>2</sub>O. The plants were watered every two days with Nitch nutrient solution diluted 1:4 with distilled water.

## *1.2. Results and Discussion*

### *1.2.1. Germination*

The effects of the three DOM samples on the seeds of slickspot peppergrass after 15-days germination are shown in Fig. 1. Numerical data of the effects of the three DOM samples examined at three different dilutions on the germination parameters of slickspot peppergrass are shown in Tables 2 and 4 (absolute germination percentage after 7 and 15 days) and Table 3 (absolute length, in cm, of primary shoot and root of germinated seeds after 7 days) and Figs. 2, 3 and 4 (same parameters expressed as percentages of those of the control treatment, assumed 100%).

In general, after a period of 7 days, the percentage of germination ranged from a minimum of 37.3% in the treatment MC-DOM 1:2 to a maximum of 111.8% in the treatments GC and GCC-DOM 1:10 (Table 2 and Fig. 2). One-way Analysis of Variance of germination data showed the existence of a significant difference for the treatments MC-DOM 1:5 (0.05 P), GC-DOM 1:5 and 1:2 (0.01 P), and MC-DOM 1:2 (0.001 P) with respect to the control (Table 2 and Fig. 2). In general, the three DOM samples at the lowest dilution exerted a negative effect on seed germination, with respect to the control. In particular, numerical data in Table 3 and Fig. 3 suggest that GC-DOM and MC-DOM at the lowest dilution exerted a significant inhibition of primary root growth (68.5% for GC-DOM 1:2 and 59.3% for MC-DOM 1:2), with respect to the control.

After 15 days, the percentage of germination ranged from a minimum of 77.4% in the treatment GC-DOM 1:2 to a maximum of 107.5% in the treatments GC and GCC-DOM 1:10 (Table 4 and Fig. 4). One-way Analysis of Variance of germination data showed the existence of a significant difference for the treatments MC-DOM 1:2 (0.05 P), and GC-DOM 1:2 (0.01 P) with respect to the control (Table 4 and Fig. 4). In general, the values of germination percentage produced by the three DOM samples at three dilutions after 15 days of germination were more similar to the control, with respect to those obtained after only 7 days.

### *1.2.2. Early-growth*

The effects of the three DOM samples on the growth of slickspot peppergrass in a commercial growing substrate after 60 days are shown in Figs. 5, 6 and 7. Numerical data of the effects of the three DOM samples examined at three different dilutions on

the growth of shoots and roots of slickspot peppergrass measured after 15 and 30-day growth are shown in Tables 5 and 6 (absolute length, in cm, of shoots and roots) and Figs. 8 and 9 (same parameters expressed as percentages of those of the control treatment, assumed 100%). Numerical data in Table 5 and Fig. 8 suggest that only GCC-DOM at the lowest dilution exerted a significant inhibition of shoots growth (83.3%), with respect to the control. After 30 days, statistical treatment of data did not show any significant difference between any treatment and the control (Table 6 and Fig. 9).

The effects of MC-DOM at different dilutions and of the three DOM samples at the lowest dilution on the growth of slickspot peppergrass in a commercial growing substrate after 6 months are shown in Figs. 10 and 11, respectively. After 6-month growth, the plants treated with the different DOM showed better growth and health conditions with respect to the control that resulted highly depressed (Fig. 10). At the lowest dilution, the maximum benefit for plant growth was produced by GC-DOM treatment with respect to the other two samples (Fig. 11), whereas MC-DOM and GCC-DOM showed the best effects at the highest dilution.

## 2. Relationship between germination and early-growth data and some compositional, structural and functional characteristics of DOM fractions

### *2.1. Experimental*

The mean values obtained for the germination percentage after 7 and 15 days, the lengths of primary shoot and root after 7 days, and of roots and shoots after 15 and 30 days were statistically correlated to a number of chemical and functional properties (Table 1) of the DOM samples examined, including pH and EC values, total organic carbon (TOC) content,  $\epsilon_{280\text{nm}}$  value,  $E_4/E_6$  ratio, and humification index (HI).

### *2.2. Results and discussion*

The correlation coefficients calculated between some DOM properties and the average of variations (%) of germination parameters after 7 days, germination percentage after 15 days, and shoots and roots of plants grown for 15 and 30 days are reported respectively in Table 7, 8, 9 and 10.

After 7-days growth significant positive correlations were apparent between: (a) the pH value and germination percentage at the average dilution ( $P \leq 0.01$ ); the TOC content

and primary root length at 1:10 dilution ( $P \leq 0.05$ ); and (c) HI and primary root length at the average dilution ( $P \leq 0.05$ ) and at 1:10 dilution ( $P \leq 0.01$ ) (Table 7).

In the case of germination percentage after 15 days, significant positive correlations were apparent between TOC content and HI and DOM at 1:2 dilution with  $P \leq 0.05$  and  $P \leq 0.01$ , respectively (Table 8). In the case of growth parameters after 15 and 30 days (Figs. 9 and 10), the only significant positive correlation was apparent between pH value and shoots length at 1:10 dilution ( $P \leq 0.05$ ) (Table 10).

## (2) RESEARCH PLANS FOR REMAINDER OF THE CONTRACT PERIOD

For the 3<sup>rd</sup> year of the contract period, research plans are the following:

- (a) Experiments on germination and early growth of pre-treated peppergrass seeds in the presence of each compost
- (b) Correlation of the germination and seedling growth data obtained with chemical and physico-chemical parameters of the composts examined, in order to find out the parameters that influence germination and growth of peppergrass.
- (c) Extension of experiments to different populations of switchgrass with known seed dormancy and germination efficiency.

## (3) SIGNIFICANT ADMINISTRATIVE ACTIONS DURING THE PERIOD REPORTED: NONE.

## (4) ANY OTHER INFORMATION : NONE.

## (5) ANNEX

- (A) AMOUNT OF UNUSED FUNDS REMAINING ON THE CONTRACT AT THE END OF THE PERIOD COVERED BY THE REPORT: .....
- (B) IMPORTANT PROPERTIES ACQUIRED WITH CONTRACT DURING THIS PERIOD: NONE.
- (C) METHOD OF REPRODUCTION: E-MAIL ATTACHMENTS, PHOTOCOPYING.

Table 1. Some chemical and spectroscopic parameters of DOM samples examined.

Sample Origin	pH	EC (dS/m)	TOC (mg L <sup>-1</sup> )	$\epsilon_{280\text{nm}}$ (L cm <sup>-1</sup> mol <sup>-1</sup> )	E <sub>4</sub> /E <sub>6</sub> ratio	Humification index (HI)
GC	7.4	3.14	140	26.8	3.6	124
MC	7.0	3.28	186	32.5	4.3	141
GCC	8.3	1.66	375	38.9	6.9	261

Table 2. Effect of DOM samples at different dilutions on seed germination (percentage of germinated seeds  $\pm$  standard error for three replicates) of slickspot peppergrass measured after 7 days.

Treatment		
Control (Nitch nutrient solution)		85.0 $\pm$ 4.71
GC-DOM	1:10	95.0 $\pm$ 2.36
	1:5	40.0 $\pm$ 7.07 **
	1:2	43.3 $\pm$ 4.91 **
MC-DOM	1:10	65.0 $\pm$ 6.24
	1:5	58.3 $\pm$ 7.20 *
	1:2	31.7 $\pm$ 4.91 ***
GCC-DOM	1:10	95.0 $\pm$ 2.36
	1:5	85.0 $\pm$ 10.27
	1:2	56.7 $\pm$ 9.53



Table 3. Effect of DOM samples at different dilutions on the length (cm  $\pm$  standard error for three replicates) of primary shoot and primary root of germinated seeds of slickspot peppergrass after 7 days.

Treatment	Shoot	Root
Control (Nitch nutrient solution)	$0.85 \pm 0.75$	$1.09 \pm 0.56$
GC-DOM 1:10	$0.74 \pm 0.31$	$0.95 \pm 0.29$
1:5	$0.69 \pm 0.40$	$0.92 \pm 0.90$
1:2	$0.69 \pm 0.55$	$0.75 \pm 0.47$ *
MC-DOM 1:10	$0.66 \pm 0.24$	$0.98 \pm 0.18$
1:5	$0.65 \pm 0.51$	$1.01 \pm 0.62$
1:2	$0.70 \pm 1.23$	$0.64 \pm 0.81$ **
GCC-DOM 1:10	$0.81 \pm 0.28$	$1.19 \pm 0.62$
1:5	$0.73 \pm 0.20$	$1.09 \pm 0.84$
1:2	$0.67 \pm 1.05$	$0.81 \pm 0.72$

Table 4. Effect of DOM samples at different dilutions on seed germination (percentage of germinated seeds  $\pm$  standard error for three replicates) of slickspot peppergrass measured after 15 days.

Treatment	
Control (Nitch nutrient solution)	$88.3 \pm 2.72$
GC-DOM 1:10	$95.0 \pm 2.36$
1:5	$78.3 \pm 2.72$
1:2	$68.3 \pm 2.72$ **
MC-DOM 1:10	$80.0 \pm 2.36$
1:5	$86.7 \pm 5.44$
1:2	$70.0 \pm 6.24$ *
GCC-DOM 1:10	$95.0 \pm 2.36$
1:5	$91.7 \pm 4.91$
1:2	$80.0 \pm 8.50$

Table 5. Effect of DOM samples at different dilutions on the length (cm  $\pm$  standard error for three replicates) of shoots and roots of slickspot peppergrass measured after 15-day growth.

<b>Treatment</b>	<b>Shoot</b>	<b>Root</b>
Control (Nitch nutrient solution)	1.22 $\pm$ 0.21	1.49 $\pm$ 0.60
GC-DOM 1:10	1.19 $\pm$ 1.28	1.34 $\pm$ 1.13
1:5	1.15 $\pm$ 0.62	1.60 $\pm$ 1.18
1:2	1.07 $\pm$ 0.55	1.32 $\pm$ 0.52
MC-DOM 1:10	1.14 $\pm$ 0.86	1.62 $\pm$ 0.54
1:5	1.06 $\pm$ 0.65	1.97 $\pm$ 2.41
1:2	1.09 $\pm$ 0.55	1.26 $\pm$ 3.06
GCC-DOM 1:10	1.16 $\pm$ 0.16	1.45 $\pm$ 0.58
1:5	1.18 $\pm$ 0.15	1.55 $\pm$ 1.44
1:2	1.02 $\pm$ 0.24 ***	1.33 $\pm$ 1.49

Table 6. Effect of DOM samples at different dilutions on the length (cm  $\pm$  standard error for three replicates) of shoots and roots of slickspot peppergrass measured after 30-day growth.

<b>Treatment</b>	<b>Shoot</b>	<b>Root</b>
Control (Nitch nutrient solution)	1.33 $\pm$ 0.36	4.27 $\pm$ 3.67
GC-DOM 1:10	1.24 $\pm$ 0.39	3.04 $\pm$ 3.64
1:5	1.20 $\pm$ 1.13	4.35 $\pm$ 6.10
1:2	1.39 $\pm$ 1.67	4.39 $\pm$ 0.90
MC-DOM 1:10	1.15 $\pm$ 0.39	5.14 $\pm$ 1.12
1:5	1.20 $\pm$ 0.38	4.81 $\pm$ 5.70
1:2	1.17 $\pm$ 0.56	3.37 $\pm$ 0.34
GCC-DOM 1:10	1.41 $\pm$ 0.14	3.96 $\pm$ 4.43
1:5	1.38 $\pm$ 0.75	4.38 $\pm$ 0.47
1:2	1.16 $\pm$ 0.69	3.46 $\pm$ 1.02

Table 7. Correlation coefficients calculated between the germination parameters of slickspot peppergrass measured after 7 days, expressed as the absolute average of variations (%), and some properties of DOM samples.

DOM properties <sup>b</sup>	Average <sup>a</sup>			DOM 1:10			DOM 1:5			DOM 1:2		
	Primary shoot	Primary root	Germinated seeds (%)	Primary shoot	Primary root	Germinated seeds (%)	Primary shoot	Primary root	Germinated seeds (%)	Primary shoot	Primary root	Germinated seeds (%)
pH	++ <sup>d</sup>	+	<b>1.000**</b>	++	+		++			--	+	++
EC		--- <sup>c</sup>	--		--					+		-
TOC		+++			<b>0.998*</b>			+	++			
ε <sub>280nm</sub>		+			+			+++	+++			
E <sub>4</sub> /E <sub>6</sub>		++			+++			+	++			
HI		<b>0.998*</b>	+		<b>1.000**</b>				++			

<sup>a</sup> Average of variations (%) obtained at dilutions of 1:10, 1:5 and 1:2

<sup>b</sup> Data in Table 1

<sup>c</sup> Negative correlation coefficient: (-) absolute value > 0.90, (--) > 0.95, and (---) > 0.99

<sup>d</sup> Positive correlation coefficient: (+) > 0.90, (++) > 0.95, and (+++) > 0.99

\* Statistically significant at  $P \leq 0.05$

\*\* Statistically significant at  $P \leq 0.01$

Table 8. Correlation coefficients calculated between the germination percentage of slickspot peppergrass measured after 15 days, expressed as the absolute average of variations (%), and some properties of DOM samples.

DOM properties <sup>b</sup>	Average <sup>a</sup>	DOM 1:10	DOM 1:5	DOM 1:2
	Germinated seeds (%)			
pH	++ <sup>d</sup>			+
EC	--- <sup>c</sup>			--
TOC	+			<b>0.998*</b>
ε <sub>280nm</sub>			++	+
E <sub>4</sub> /E <sub>6</sub>	+			+++
HI	++			<b>1.000**</b>

<sup>a</sup> Average of variations (%) obtained at dilutions of 1:10, 1:5 and 1:2

<sup>b</sup> Data in Table 1

<sup>c</sup> Negative correlation coefficient: (-) absolute value > 0.90, (--) > 0.95, and (---) > 0.99

<sup>d</sup> Positive correlation coefficient: (+) > 0.90, (++) > 0.95, and (+++) > 0.99

\* Statistically significant at  $P \leq 0.05$

\*\* Statistically significant at  $P \leq 0.01$

Table 9. Correlation coefficients calculated between growth parameters of slickspot peppergrass measured after 15 days, expressed as the absolute average of variations (%), and some properties of DOM samples.

DOM properties <sup>b</sup>	Average <sup>a</sup>		DOM 1:10		DOM 1:5		DOM 1:2	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
pH								
EC								
TOC							-	
$\epsilon_{280\text{nm}}$			+				---	
$E_4/E_6$							-	
HI								

<sup>a</sup> Average of variations (%) obtained at dilution of 1:10, 1:5 and 1:2

<sup>b</sup> Data in Table 1

<sup>c</sup> Negative correlation coefficient: (-) absolute value > 0.90, (--) > 0.95, and (---) > 0.99

<sup>d</sup> Positive correlation coefficient: (+) > 0.90, (++) > 0.95, and (+++) > 0.99

Table 10. Correlation coefficients calculated between growth parameters of slickspot peppergrass measured after 30 days, expressed as the absolute average of variations (%), and some properties of DOM samples.

DOM properties <sup>b</sup>	Average <sup>a</sup>		DOM 1:10		DOM 1:5		DOM 1:2	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
pH			<b>0.999*</b>		<sup>d</sup>			
EC			-- <sup>c</sup>		---			
TOC					++			
ε <sub>280nm</sub>								
E <sub>4</sub> /E <sub>6</sub>					++			
HI					+++			

<sup>a</sup> Average of variations (%) obtained at dilution of 1:10, 1:5 and 1:2

<sup>b</sup> Data in Table 1

<sup>c</sup> Negative correlation coefficient: (-) absolute value > 0.90, (--) > 0.95, and (---) > 0.99

<sup>d</sup> Positive correlation coefficient: (+) > 0.90, (++) > 0.95, and (+++) > 0.99

\* Statistically significant at  $P \leq 0.05$

\*\* Statistically significant at  $P \leq 0.01$

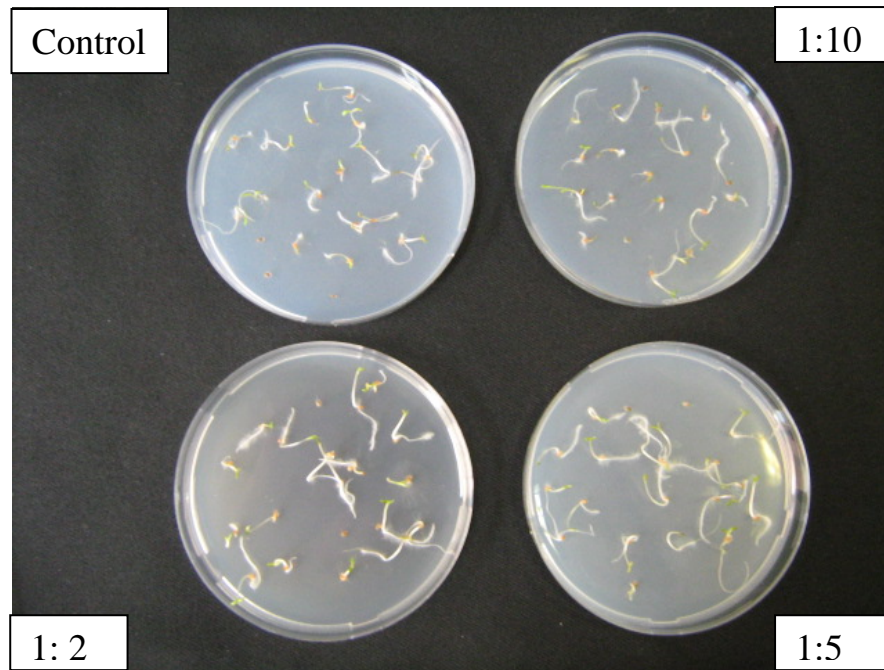
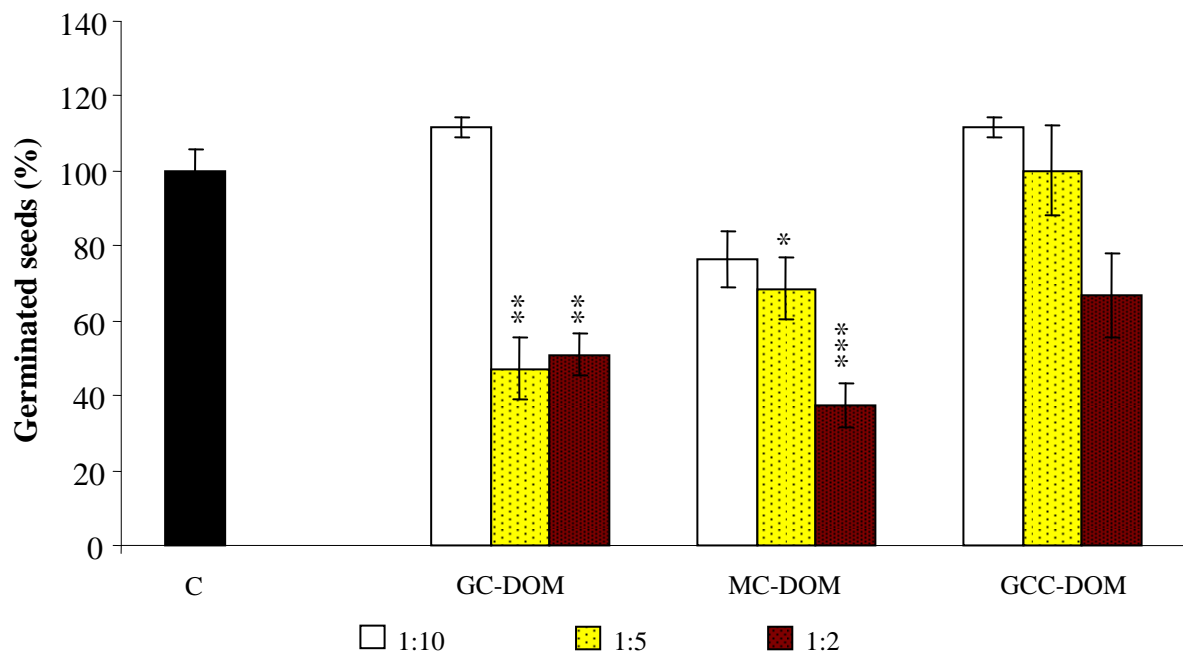
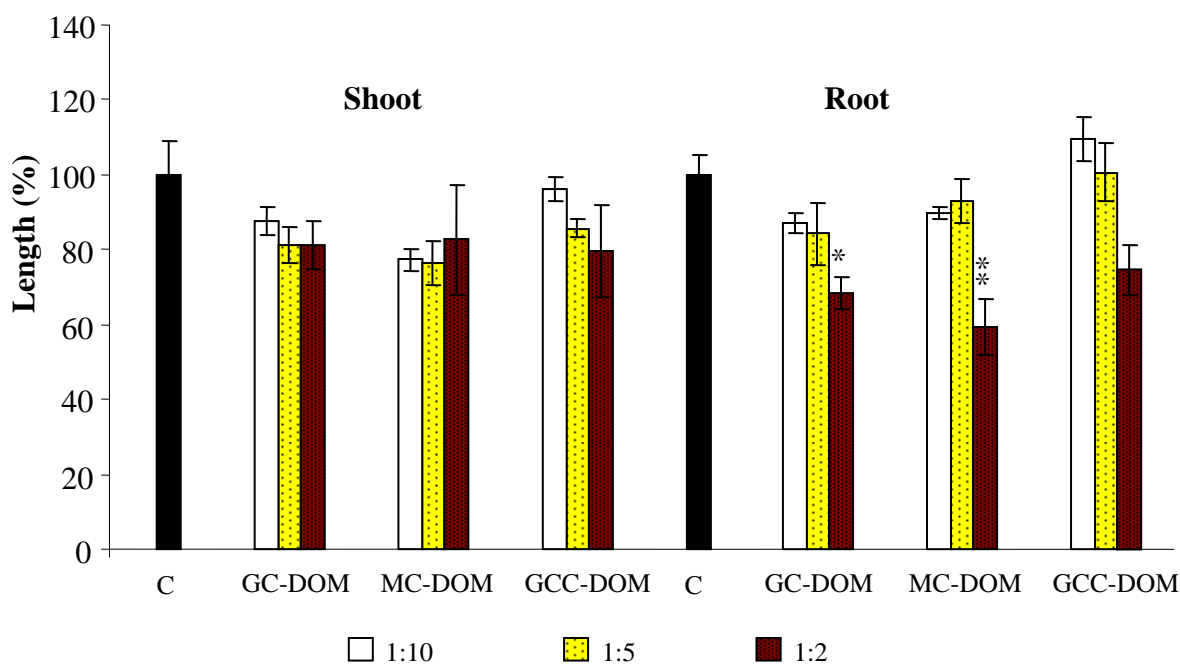


Figure 1. Effect of MC-DOM at different dilutions on the germination of slickspot peppergrass in agar substrate.



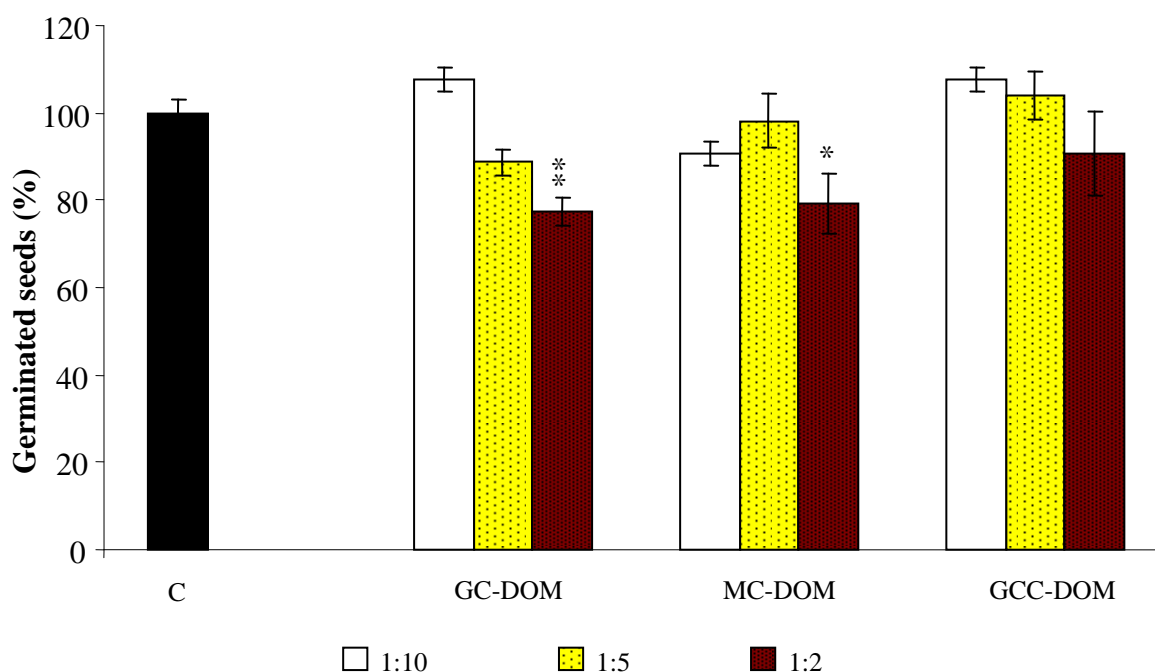
\*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$ , according to the LSD test

Figure 2. Effect of DOM samples at different dilutions on the number of germinated seeds after 7 days expressed as percentages of control treatment (100 %). The vertical line on each bar indicates the standard error for 3 replicates.



\*  $P \leq 0.05$ ; \*\* $P \leq 0.01$ , according to the LSD test.

Figure 3. Effect of DOM samples at different dilutions on primary shoot and root length of germinated seeds, expressed as percentages of control treatment (100 %). The vertical line on each bar indicates the standard error for 3 replicates.



\*  $P \leq 0.05$ ; \*\* $P \leq 0.01$ , according to the LSD test.

Figure 4. Effect of DOM samples at different dilutions on the number of germinated seeds expressed as percentages of control treatment (100 %), measured after 15-days. The vertical line on each bar indicates the standard error for 3 replicates.



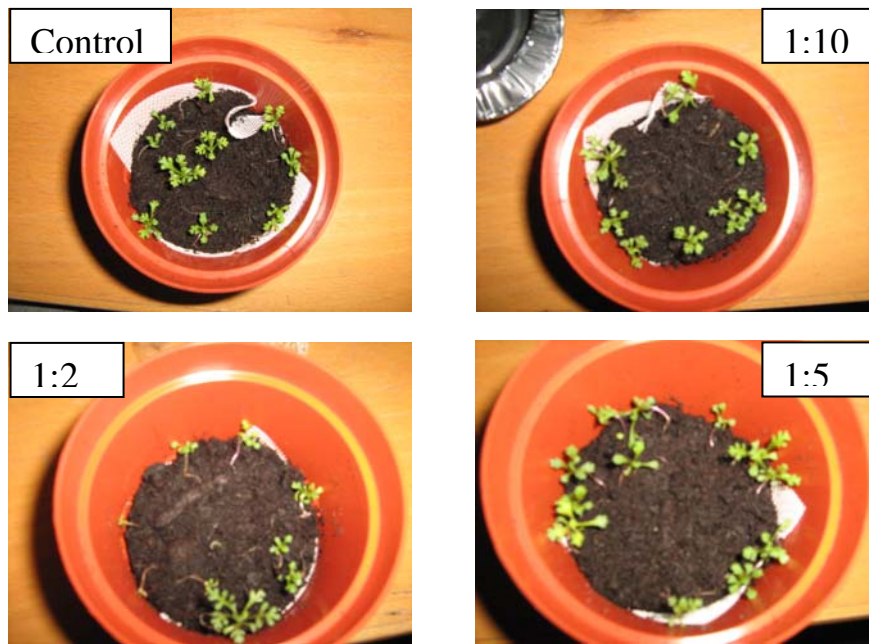


Figure 5. Effect of GC-DOM at different dilutions on the growth of slickspot peppergrass in a commercial growing substrate after 60 days.

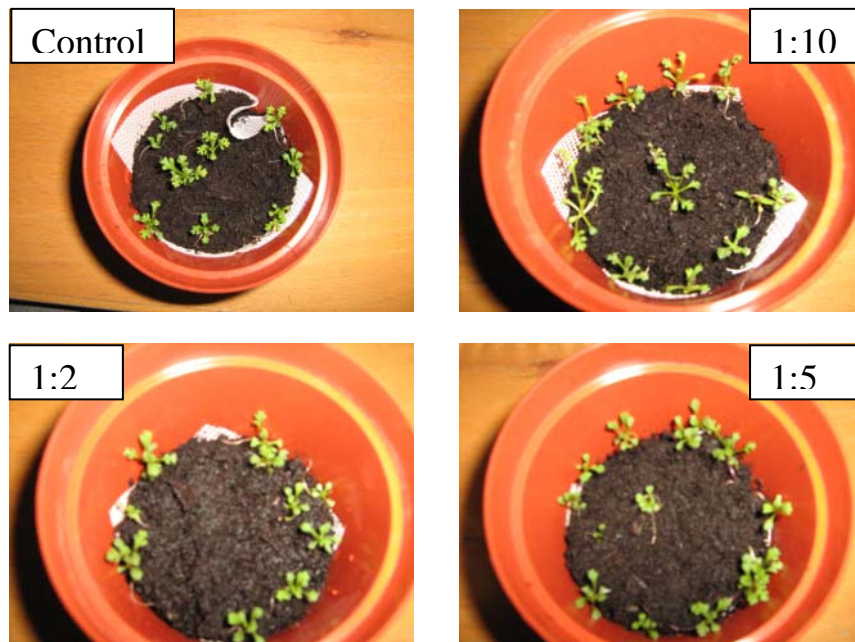


Figure 6. Effect of MC-DOM at different dilutions on the growth of slickspot peppergrass in a commercial growing substrate after 60 days.

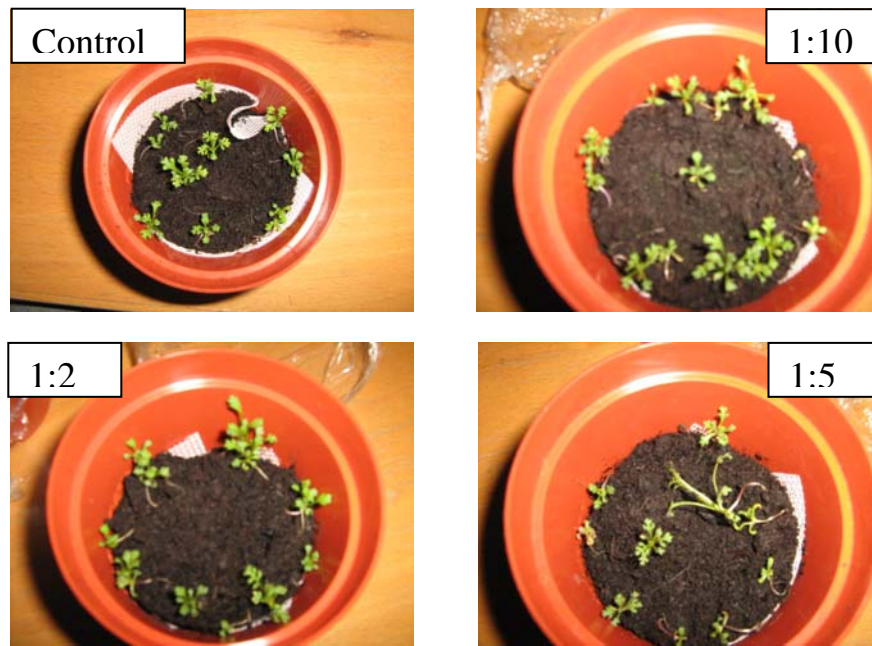
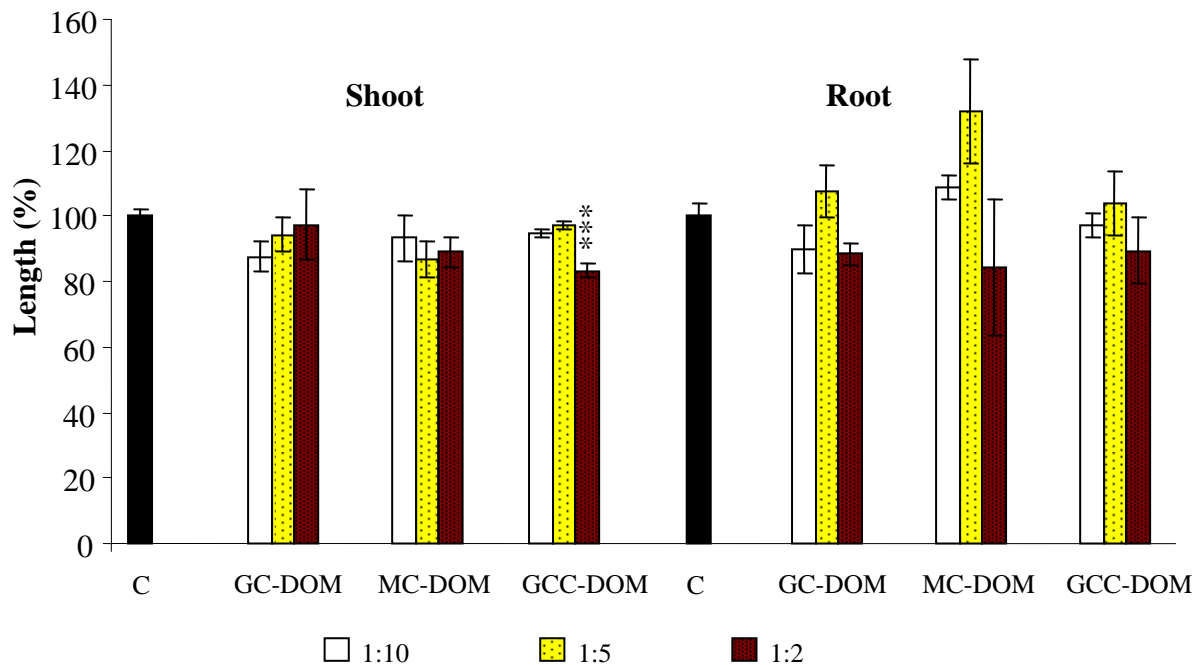


Figure 7. Effect of GCC-DOM at different dilutions on the growth of slickspot peppergrass in a commercial growing substrate after 60 days.



\*\*\* $P \leq 0.001$ , according to the LSD test.

Figure 8. Effect of DOM samples at different dilutions on the length of shoots and roots expressed as percentages of control treatment (100 %) measured after 15-day growth. The vertical line on each bar indicates the standard error for three replicates.

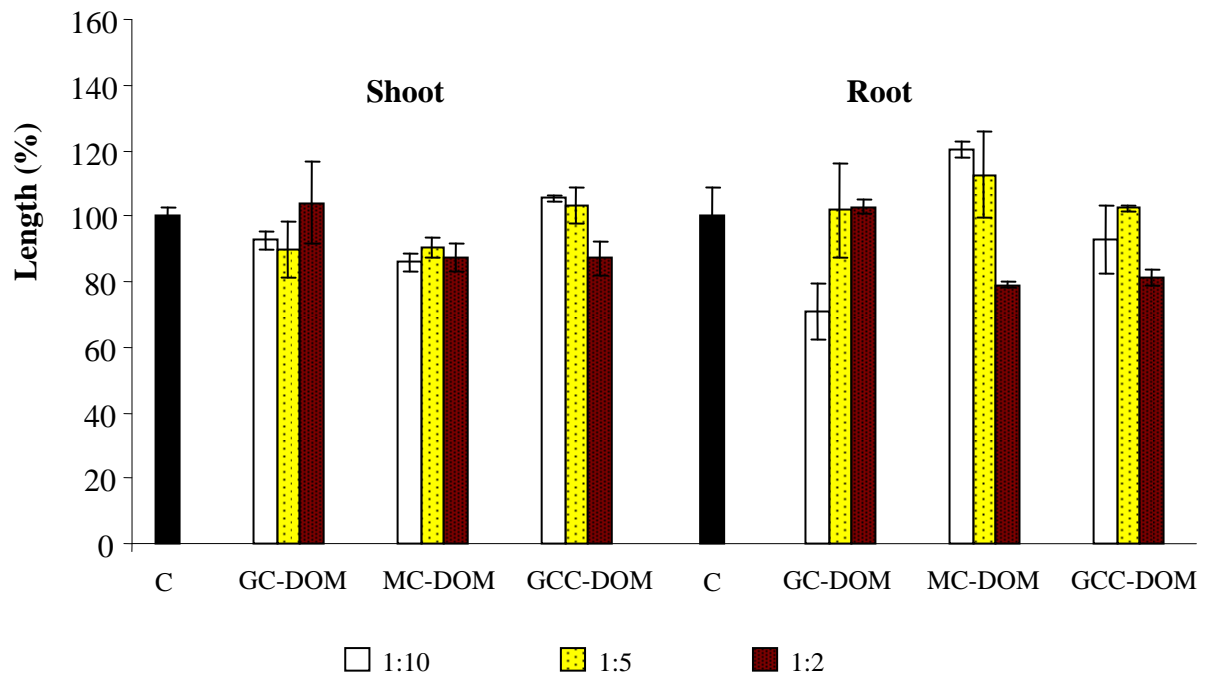


Figure 9. Effect of DOM samples at different dilutions on the length of shoots and roots expressed as percentages of control treatment (100 %) measured after 30-day growth. The vertical line on each bar indicates the standard error for three replicates.

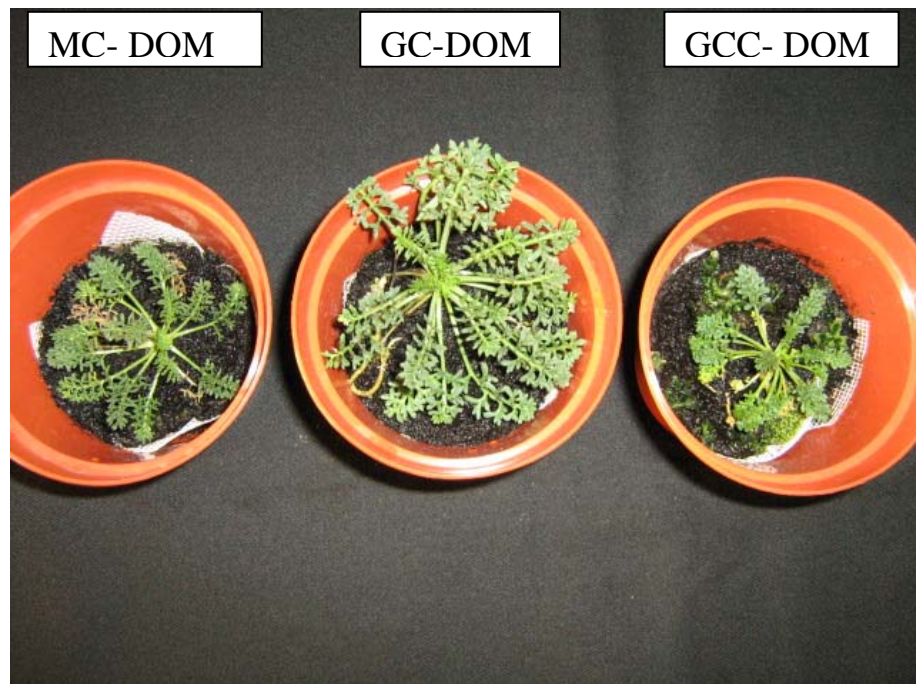


Figure 10. Effect of different origin of DOM at the lower dilution on the growth of slickspot peppergrass after 6 months.



Figure 11. Effect of MC-DOM at different dilutions on the growth of slickspot peppergrass after 6 months.